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A STUDY OF THE BIOLOGICAL ACTIVITY OF ERIONITE IN RELATION TO ITS CHEMICAL AND STRUCTURAL CHARACTERISTICS

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ABSTRACT

Fibrous erionite appears to have many times the mesothelioma induction potential of asbestos. A member of a class of minerals termed zeolites, it differs markedly from asbestos in many physico-chemical characteristics. Zeolites are uniquely powerful in ion exchange, adsorption of small molecules, and catalytic activity. The hallmark of the zeolites is the presence of spaces or voids in the internal structure which communicate with the exterior by means of minute pores with effective diameters of only a few A. The critical kinetic diameter of these pores for erionite is 3.6A x 5.2A. The interior spaces are larger. These voids in the structure are brought about by the mode of stacking of the aluminum and silica tetrahedra with shared oxygen atoms which differ in the various zeolites. Additionally, cations are associated with the zeolite structure, most commonly Na^{+}, K^{+}, and Mg^{+2} and Ca^{+2} which may occlude the entrance to the pores. Another characteristic of zeolites is the very large surface area (chiefly internal). Erionite has an internal surface area approximately 200 m^2/gram which contrasts with a total surface area for chrysotile and crocidolite of 24 m^2/gram and 8-10m^2/gram respectively. For preliminary biological studies, experiments were based on the hypothesis that the active surface sites within the internal voids are contributory to the unusual activity of this mineral. Two approaches were followed: encapsulation of isopentane into the internal spaces and exchange of cations, Cr^{+2} and Ca^{+2}. Preliminary data suggests that toxicity to V79 cells was altered by these processes. The implication of the intense tumorigenesis of erionite may be of great basic importance in contributing data for mechanistic studies such as the illumination of a second variable in tumor induction (in addition to the length-width phenomenon).

Experiments with erionite should also be of great interest in conjunction with other carcinogenic influences. Strong interaction for instance would be expected with small molecular compounds present in gases and vapors from tobacco smoke and thus erionite (or other zeolites) might have either positive or negative effects on the biological activity of these substances.

INTRODUCTION

The recognition that the silicate mineral, erionite, induces mesothelial tumors similar to asbestos is attributable to the notable observations of Baris of Ankara, Turkey. His observations were made extending over a number of years in certain small Turkish villages in the province of Cappodocia where there was an extremely high incidence of mesothelioma in the absence of obvious contact with commercial asbestos (1). Dr. Baris drew international attention to this discrepancy and minerological studies were carried out. Pooley noted that the mineral erionite was ubiquitous in certain of these villages - its source being the volcanic tuff (2) (3). Other studies, however, detected the presence of small amounts of tremolite and chrysotile asbestos (4). Soon after these observations, experimental animal studies demonstrated that fibrous erionite from Turkish and North American sources was more tumorigenic on a per mass basis than asbestos on the basis of intrapleural inoculation (5). When similar material was used in inhalation experiments in rats, only mesothelioma was induced. This was in striking contrast to all previous experiments employing asbestos where mesotheliomas were of minor importance as compared to pulmonary carcinoma (6).

Similar predominance of mesothelioma had previously been noted in the Turkish data, Figure 1, Table 1, (7). Intrapleural inoculation data from our laboratory strongly corroborates the vastly more potent mesothelioma induction attributable to erionite as compared to asbestos when the dose was computed by the number of categorized fibers inoculated (8). Preliminary analysis of data from an intracheal study underway in our laboratory also demonstrates a similar preponderance of mesothelioma induction by erionite (9), Table 1.

It would thus appear evident that the disease reported from Turkey was due to the exposure to erionite. It also seems clear that this mineral has a greater potency to induce mesothelioma in both human beings and experimental exposure of rats than asbestos. Despite these findings, negative experiments with non fibrous erionite indicate that even with this mineral, a fibrous structure appears essential for mesothelioma induction (5). The facinating history of solution of the Turkish endemic focus has been recently reviewed by Baris (10).

Minerology of Erionite

In view of the high potency of erionite for mesothelial induction, it would seem of value to examine its special physical and chemical properties to determine which of these may explain the far greater potential for mesothelial tumor induction. As noted above, experiments with fibrous and non fibrous erionite indicate, that despite its minerologic peculiarities, a fibrous shape is a prerequisite for mesothelioma induction. (No biological studies on the only other fibrous zeolite, mordenite, appear in the available literature.) Though it is a silicate, other aspects of erionite differ greatly from asbestos since it belongs to a class of minerals termed zeolite which are unique crystalline hydrated alumino-silicates. Structurally, zeolites consist of a "framework" which is based on an infinitely extending three dimentional network of AlO_4 and SiO_4 tetrahedrals linked together by sharing all the oxygens. This framework contains channels and interconnecting voids termed "cages" which are occupied by cations and water molecules. The cations presented are commonly Na^+, K^+, Mg^{+2} and Ca^{+2} but many other cations may be exchanged or held.

The internal voids are interconnected and communicate to the surface via minute pores of effective diameter of only a few Å(11).

By virtue of these internal channels and cages, zeolites contain an enormous surface area per unit mass. For instance, erionite has a surface area of 200 m^2/gram compared to 10m^2 gram for crocidolite asbestos. Importantly, nearly all the surface for zeolite is internal while for asbestos, it is external. The large internal surface furnishes a great number of sequestered active sites. The void spaces of defined size gives zeolites (or erionite) uniquely active properties for ion exchange, adsorption of small molecules and strong catalytic properties. Thirty-four natural zeolites have been reported and more than 100 have been synthesized from silica gel. These unique physico-chemical properties of zeolite have been exploited widely for chemosorption of small molecules, ion exchange and catalysis. Naturally occurring zeolites are mined for use as soil amendments for agriculture, removal of radioactive wastes, administered as additives to stock feed and used as adsorbants for waste water treatment, oxygen enrichment and many other purposes (11) (12). Zeolites have been synthesized with special properties for specific purposes, such as refining of petroleum where adsorption of specific hydrocarbons in specially tailored synthetic zeolites enables catalytic cracking of the smaller compounds with enhanced production of gasoline with higher octane ratings. From the standpoint of tumorigenesis, fibrous erionite (and possibly fibrous mordenite*) are of interest for two reasons: (1) the possibility that human health effects similar to that of the Turkish villages may occur elsewhere and thus constitute a health problem in other countries; and (2) the

determination of the mechanisms responsible for induction of tumors might provide insight into the causation of tumors by mineral fibers in general.

Possible Epidemiologic Consideration

What are the possibilities for health risks to erionite in the United States? In the Turkish microfoci, the presence of the mineral widely dispersed in nearly every niche in the environment-rock, soil, stucco, etc. coupled with a close-to-the-earth lifestyle of the population (assuring exposure from the cradle to the grave) strongly favor human risk to tumor induction. In the case of the United States, erionite has been reported as occurring in rather pure form in a number of arid sites in the Great Basin or Rocky mountain area - Oregon, South Dakota, Nevada, and Arizona in sedimentary volcanic rock, chiefly, tuff. It also has been reported in similar sites as a contaminant with other zeolites, clinoptilolite for instance (Figure 2). It is likely that a detailed geological survey would reveal other sources present in deposits of tuff throughout areas in the western United States (13).

It is perhaps fortunate, as far as human risks are concerned, that these areas in the United States are sparsely populated yielding few possibilities for human contact. By the same token the low population would make the application of epidemiology studies difficult. While mesotheliomas have been reported from the Rocky mountain region where asbestos contact would be expected to be minimal (14), no case control studies have been conducted to sort out such cases on the basis of association with erionite localities or contact with asbestos. Another source of contact with erionite must be considered. That is the accidental exposure by the presence of contaminating erionite fibers in other natural zeolites such as clinoptilolite

which are mined for use in various agricultural or pollution control activities. This fact would argue for careful analysis in order to detect the presence of possible contaminating fibrous erionite or mordenite before widespread commercial application of such zeolites was undertaken.

Value of Erionite for Mechanism Studies

The most obvious difference between erionite and asbestos is the presence of the internal voids in the zeolite. Thus it appears reasonable to postulate that some mechanism associated with these voids might be contributing to its potency. Possible mechanisms might be increased free radical emission from interaction with the numerous enclosed oxygen sites, exchange of an ion required as a building block for a compound necessary in a cellular defense against carcinogenic influence or possibly some catalytic activity similar to that utilized for petroleum cracking (15).

For instance, it has been speculated that in petroleum the superior activity of zeolite in comparison to aluminosilica is due to a combination of three effects (1) greater concentrations of active sites; (2) greater concentration of hydrocarbons in vicinity of the active sites; and 3) the electric field within the zeolite voids might polarize C-H bonds and amplify the carbonium ion mechanism (16). Most of these conditions are reported in the petroleum cracking; whether similar conditions would obtain in the animal body is unknown although interaction with NH_4^+ is a possibility.

Regardless of the validity of such mechanisms, it is usually thought that the pore plays an essential role in the unusual chemical activity of zeolite.

Work to test the above hypothesis was approached as follows:

1) Blockage of the pores and void spaces by encapsulation of isopentane and exchange of Cr^{+2} and Ca^{+2} (17) and measuring the biological effect by *in vitro* cytotoxicity testing via V79 cells. The V79 system has shown a statistical correlation with mesothelial tumor induction (18).

2) Determination of ion exchange by peritoneal implantation of the mineral contained in sealed diffusion tubes in the peritoneal cavity of rats.

3) Measurement via spin magnetic resonance of radical emission after treatment of the mineral with various substrates present in animal tissues.

4) Determination of radical emission by reaction with V79 cells *in vitro* when the mineral was contained in sealed tubes as in (2) above. In all instances, these reactions would be compared to similar treatments with crocidolite, chrysotile, asbestos and attapulgite (16).

The encapsulation process was carried out briefly as follows:

Known amounts of the mineral to be encapsulated were introduced into a system and subjected to high vacuum less than 10^{-5} torr and heated to 350-400°C for twelve hours to remove residual gases, surface impurities and water of hydration. Subsequently the encapsulation of purified isopentane was accomplished in the same system by its introduction into the system and exposure to pressure of 2000 torr and temperature of 400°C for two hours. Isopentane was chosen for the encapsulation because at room temperature, its critical kinetic diameter is slightly larger than the pore size of the erionite but can be forced through the pores by increased pressure and temperature. Successful encapsulation was demonstrated via infrared spectroscopy. It did not deencapsulate when immersed in water. See ref (16) for details of these procedures. Ion exchange was carried out by exposure of the purified fibers to chromium or calcium chloride.

Preliminary results of the encapsulation and ion exchange studies are included in Table 3. While the data for reduction of the biologic effect by encapsulation of isopentane and its augmentation by exchange of Ca^{+2} are statistically significant, these studies should be considered only as preliminary probes to be repeated and compared to duplicates performed with other minerals such as asbestos and attapulgite before attributing significance to the findings.

DISCUSSION AND CONCLUSIONS

Mesothelioma induction by erionite differs from that of asbestos in at least three aspects: (1) greater number of tumors induced by a given mass or number of mineral fibers; (2) shorter induction period and 3) greater tendency to induce pleural mesothelioma rather than lung tumors when exposure is via the inhalation or intratracheal routes where tumorigenesis by erionite is induced by far fewer fibers than asbestos. Since the size and the shape of erionite mineral fibers are not particularly different than asbestos, it would appear clear that some other factor or factors (in addition to length and width of the fibers) must play a determinate role in erionite tumorigenesis. The zeolites (including erionite) have unique physico-chemical properties due to the void spaces within the crysotile lattice which provide opportunities for intense physical and chemical interactions with chemical compounds. These are exchange of cations, cation complexes and adsorption of small molecules. Under certain conditions, zeolites interact with NH_4^+ probably by a chain of reactions via active sites within the voids with the promotion of reaction protonation or election transfer or the ability to donate or accept protons. Whether these mechanisms would be operating to foster carcinogenesis is at this time not determined. However, extension of

the preliminary encapsulation and cation exchange studies maintained above would seem to be indicated. Additionally, it might be fruitful to pursue the potential ion exchange and/or molecular adsorption after implantation within the animal body. The utilization of the hollow fiber membranes to contain the mineral fibers would allow recovery and possible analysis after in vivo exposure. The hollow fiber membrane tubes allow molecular weight materials of up to 300,000 to freely by-pass. While actual cell migration through the fibers is precluded, active biomolecules would have access. Previously, no convenient method of recovery of fibers after in vivo implantation existed.

It would seem of interest to study the interaction of zeolite with tobacco smoke where exchange with nitric oxide or ammonia and possible adsorption of small molecules present in the gas phase might be expected to alter the biological activity of the smoke either positively or negatively.

It should be recommended that further physico-chemical studies should be carried out with carcinogenic and non-carcinogenic systems. While not supported by specific experimental evidence, an alternative reasoning may have some merit. Examination of the scale of events on a molecular level opens serious questions as to our understanding of the mechanism. We know fibrous structures in the micron range are required with the correct length/diameter ratio on the same scale. Internal zeolite pore sizes are many orders of magnitude smaller than single molecules (especially biological ones). Thus due to smaller pore size biomolecules will not penetrate. While activated or catalytic effects are possible and even probable, the external surface array of biological molecules implied by the voluminious data on fibrous tumorigenesis in general, suggests a more effective arrangement on zeolites than for asbestos. One can conjecture the "templating" of high weight.

biological molecules in arrays wrapped in regular repeating sequences on the fibers, due to the "key-hole" effects of pendant groups of the biomolecules adsorbed in the pore sites in the zeolite. Thus the regularity and specificity of the zeolite structure makes available a mandrel with binding sites leading to ordered biological reaction candidates with an active 3rd body energy donor.

It would seem that the study of erionite in conjunction with asbestos by physico-chemical and molecular biological methods might open avenues for determining basic mechanism of tumorigenesis by mineral fibers. Additionally, careful epidemiological observation in selected areas where human contact is possible might reveal human cases of mesothelioma or other pleural disease in other areas where foci of this mineral occur.

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TABLE LEGENDS

Table 1. Similar to Figure 1 showing numerical data.

Table 2. Shows comparison of excess mesothelioma for erionite compared to asbestos after intratracheal administration.

Table 3. Shows results of experimental effect of encapsulation of isopentane and ion exchange with Ca^{+2} and Cr^{+3} on survival of V79 hamster lung cells *in vitro*. Note that encapulation with isopentane reduced the activity (increased colony survival) by 59.2% of control. Exchange with Ca+2 increased activity (reduced colony survival) to 9.2% of control. Control in each instance was dehydrated, degassed erionite.

FIGURE LEGENDS

Figure 1. Shows the relative proportions of lung cancer and mesothelioma for exposure to asbestos and erionite in human beings and experimentally treated rats. 1-5 cohorts industrially exposed to unspecified asbestos, 6 rats exposed by inhalation to various asbestos species, 7 human exposures to erionite in Turkish Village, 8 same but females only, and 9 inhalation exposure of rats. See Table 1 for numerical data.

Figure 2. Forty natural zeolites are known. Many of these occur in the Western USA and in scattered locations elsewhere. Fibrous erionite has been reported in thirteen locations either in pure forms as in some origin where the samples used in these experiments originated or as contaminents in other forms of zeolite - clinoptilolite for instance. Fibrous modenite also has been reported from thirteen sites. Other unreported sites are likely for both minerals. Distribution maps after Sheppard.

TABLE 1

COMPARISON OF ERIONITE TO ASBESTOS

Mortality Studies in Man and Experimental Rats

	ASBESTOS						ERIONITE		
	Cohort[1]	Cohort[2]	Cohort[3]	Cohort[4]	Cohort[5]	Rat[6]	Total[7]	Female[8]	Rats[9]
% Cancer of All Deaths	46.6	42.3	31.1	36.0	39.9	NA	61.1	69.2	96.4
% Lung Cancer of All Cancers	44.9	47.8	51.5	37.5	47.1	21.1	19.5	5.5	0
% Mesotheliomas of All Cancers	17.7	16.0	6.7	20.8	15.2	2.1	65.8	88.9	100
Lung Cancer/ Mesotheliomas	2.54	2.99	7.69	1.80	3.10	10.05	0.30	0.62	0/100

Table 2

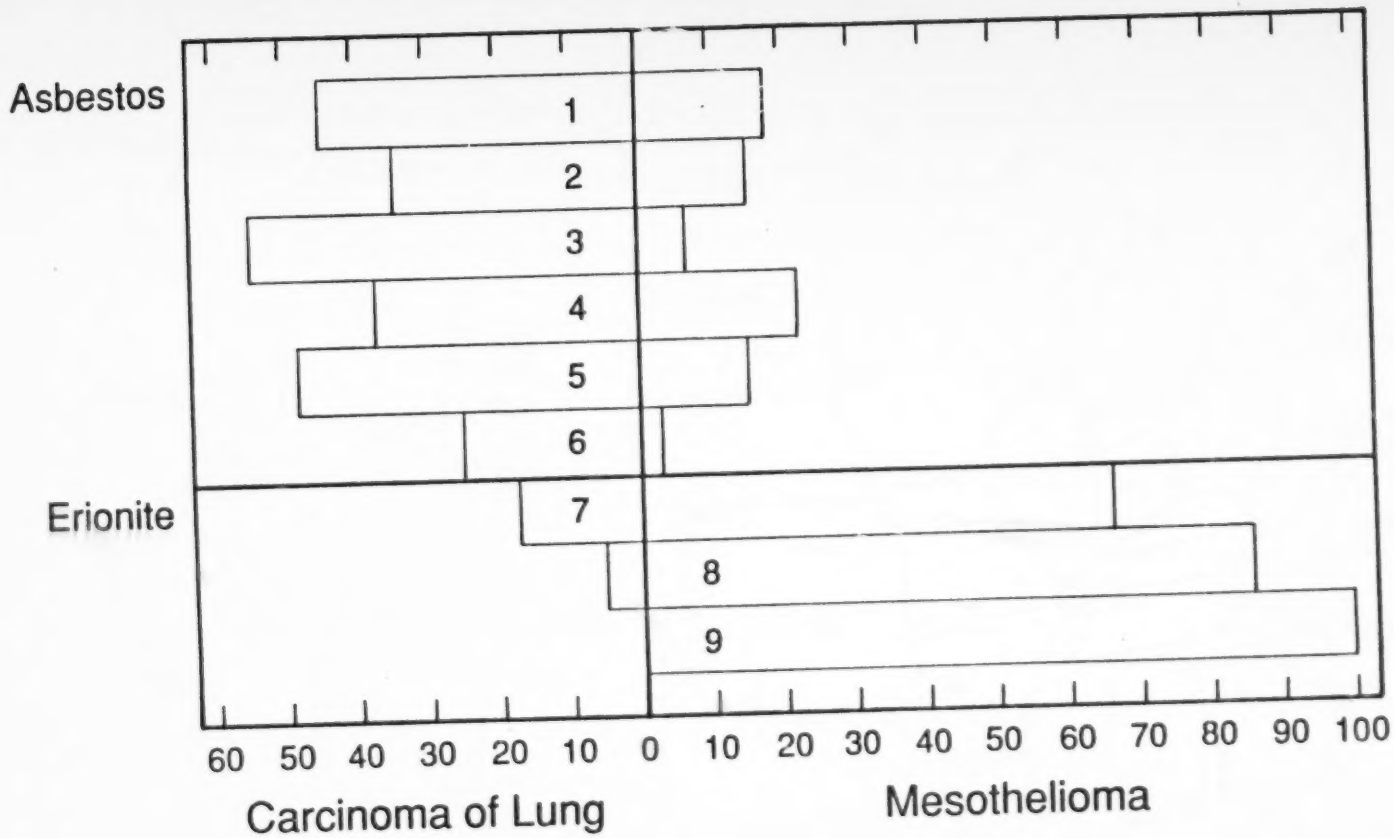
Mineral Fiber	% Tumor		Relative Number of Fibers <8 μm x >0.25 μm Unit Mass
	Lung Cancer	Mesothelioma	
Crocidolite	13	3.8	8.6
Chrysotile	32	7	1090
Erionite	4	48	3.3

TABLE 3

Sample	Plate #	# Colonies	X avg.	% Control
Control (NEG. CONTROL)	1	82		
	2	73	76	
	3	73		
Degassed	1	30		
Erionite (POS. CONTROL)	2	26	28	36.8
	3	28		
Ca^{+2} exchanged	1	5		
Erionite	2	7	7	9.2
	3	9		
Cr^{+3} exchanged	1	20		
Erionite	2	25	23.33	30.7
	3	25		
Encapsulated	1	45		
Erionite	2	33	45	59.2
	3	57		

FIGURE 1

Comparison of Erionite to Asbestos



1-5 Various cohorts (Selikoff and Lee 1978)

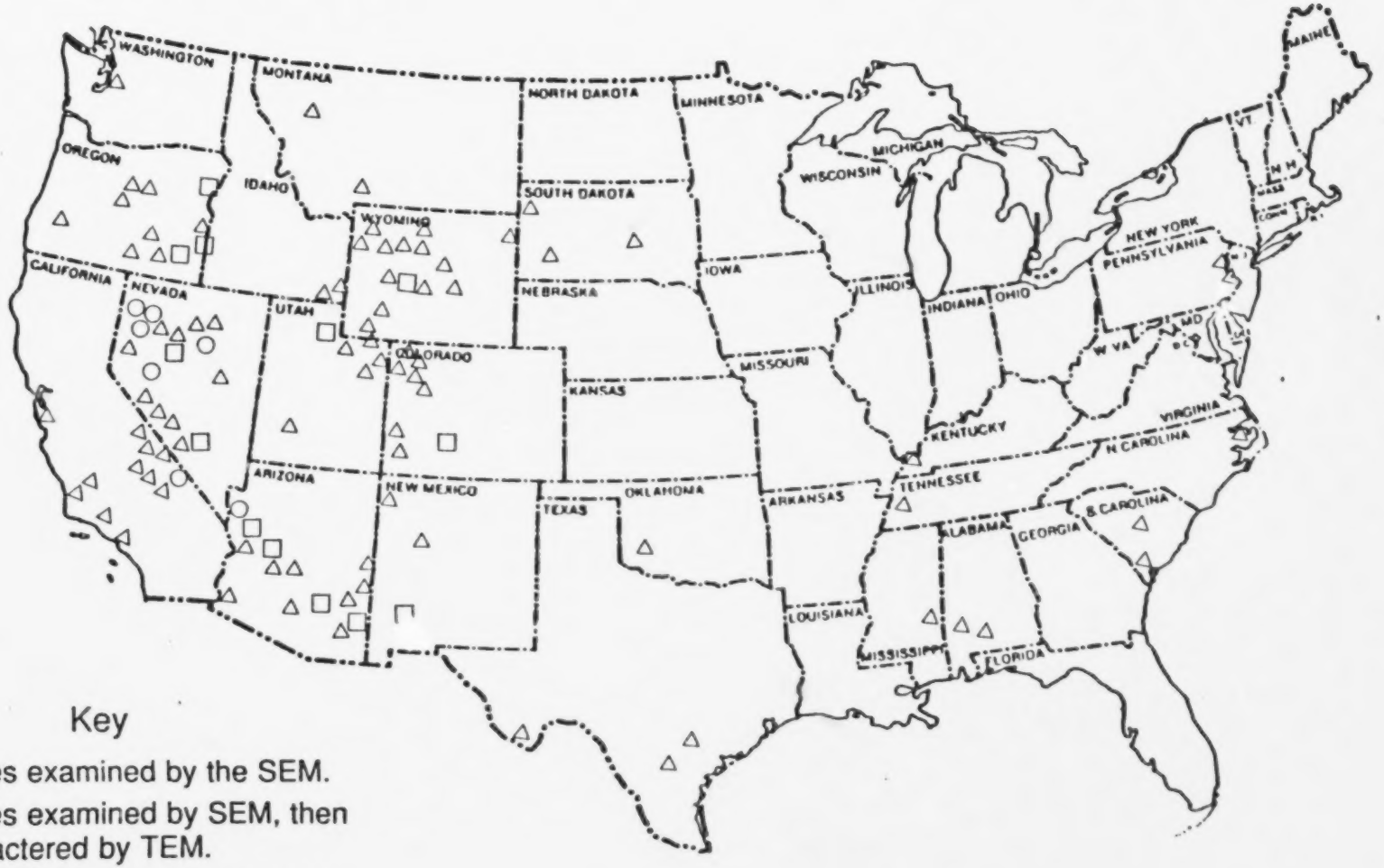
6 Wistar rats exposed via inhalation to asbestos (Wagner *et al.* 1974)

7-8 Environmental exposure in Turkish village, Tuzkoy, (Artvinli & Baris 1979)

9 Fisher rats exposed via inhalation to erionite (Wagner *et al.* 1985)

FIGURE 2

Zeolite occurrences (after Sheppard) and sample locations.



Key

□ Samples examined by the SEM.

○ Samples examined by SEM, then characted by TEM.

△ Other zeolite locations reported by the U.S. Geological Survey.